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THE MICROFLORA DEVELOPING AEROBICALLY ON BEEF AFTER AGEING IN VACUO

J T Patterson & Jane P Sutherland

Ministry of Agriculture for Northern Ireland
and the Queen's University of Belfast
Agriculture and Food Science Centre
Newforge Lane, Belfast BT9 5PX

INTRODUCTION

CONSIDERABLE INTEREST has been shown in recent years in the vacuum-packaging of primal cuts of beef, and a number of reports have appeared dealing with the microbiology of the process. Recent work in this field has been by Shaw and Nichol (1969), Taendler and Heinz (1970, a, b; 1971), Pierson, Collins-Thompson and Ordal, 1970; while Reagan, Jeremiah, Smith and Carpenter (1971), Jeremiah, Smith and Carpenter (1972) have dealt with the vacuum-packaging of lamb. Various aspects of vacuum-packaging were considered at this meeting in 1971 and 1972. The purpose of this report is to give some results which have been obtained during studies on the microbiology of vacuum-packaged beef. The aim of this part of the work has been to characterize the flora which develops on meat after the opening of the vacuum-pack, and to see whether this bears any relationship to the initial flora on the meat before packaging. In addition some preliminary results have been obtained following the inoculation of meat prior to packaging with strains of Staphylococcus aureus.

MATERIALS AND METHODS

Development of the flora during vacuum-packaging

A striploin (longissimus dorsi) was obtained from a meat processing plant from the cutting line where meat after cooling for 48 h at 1.1° was being prepared for vacuum-packaging. In the laboratory this was divided into 12 pieces. Six of these pieces were sampled immediately on both the meat and fat sides, using the method of Williams (1967) in which a sterile stainless

steel tube covering an area of 10 cm^2 was pressed on to the surface to be sampled, 10 ml of 0.5% ($^W/V$) peptone water pipetted into the tube, the surface scraped with a sterile pipette for 1 min and the liquid then withdrawn. Further dilutions were made in sterile 0.5% peptone water and 0.1 ml amounts from suitable dilutions spread on the surface of previously prepared plates of tryptone glucose yeast extract agar (TYGE). These were incubated under conditions of reduced O_2 concentration (produced by burning a candle in a sealed enclosed space) for 11-12 d at 4°. The other 6 pieces of meat were packaged individually in 80 gauge nylon/280 gauge polythene film (WVTR 5.8g/m^2/24 h/90% RH/40°C; O_2 permeability 30 cc/m^2/24 h/atmosphere/0% RH/22°C) using a Swissvac 'Popular' vacuum-packaging machine, placed in an incubator in which the temperature was maintained at $0-2^\circ$, the RH at 98% and held under these conditions for a period of 8 weeks. After this period the pieces were removed from the packages and sampled by the method described above. Measurements of pH on the initial sampling solutions were also obtained by means of a glass electrode, and of the extract release volume (ERV) of the meat using the method of Jay (1964).

Identification of isolates

A maximum of 20 isolates was obtained from plates from suitable dilutions of each of the samples by the technique of Harrison (1938) to give 120 isolates from both meat and fat surfaces, before and after storage (480 in all). The isolates were stained by the method of Gram and examined for pigmentation and colonial morphology after which Gram-negative strains were tested for the presence of catalase, oxidase, motility, dissimilation of glucose (Hugh and Leifson, 1953), ability to produce water soluble fluorescent pigment together with ability to grow at 42°. Gram-positive isolates were checked for catalase, and if rods for motility, growth on the media of Rogosa, Mitchell and Wiseman (1951), and Gardner (1966).

Microbiology of meat stored in air, after vacuum-packaging

Similar methods were used to those described above, except that the pieces of meat from the striploin (8 in number) were vacuum-packaged in the processing plant, while the initial and subsequent samplings were on meat surfaces only. The vacuum-packaged pieces of meat were stored at 0-2° for up to 10 weeks, and 1 piece was withdrawn each week commencing at the third week of storage. This was cut into 8 slices, cutting across the muscle using a sterile knife and each slice placed on a new polystyrene tray, overwrapped with clear plastic film having the following properties; WVTR 700g/m^2/24 h; O_2 transmission 8500 cc/m^2/24 h; CO_2 transmission 70,000 cc/m^2/24 h. These slices were stored in a domestic refrigerator at 4-6° for up to 96 h. Slices were withdrawn for sampling using the William's technique after intervals of 6 or 12 h. Micro-organisms on the meat were recovered using TGYE agar for total counts, the plates being incubated for 12 d at 4° and 4 d at 15°, violet red bile agar, (VRB) (Oxoid, CM107) for coliforms, incubated for 24-48 h at 30° and the medium of Masurvosky, Goldblith and Voss (1963) (MGV), for pseudomonads. The pH was determined in this experiment on surface scrapings from the meat.

Survival of Staphylococcus aureus on vacuum-packaged meat

In this investigation known numbers of two strains of Staph. aureus were inoculated on to the surface of small pieces of meat (30-50g), spread with a sterile bent glass rod, the meat was then vacuum-packaged in small pouches made from large bags of similar material to that used in the earlier experiments. These were incubated at 0-2° for up to 8 weeks, and samples withdrawn each week for investigation. Coagulase positive staphylococci were recovered from the meat by homogenising all of each piece of meat in a suitable volume of 0.5% peptone water, using the MPN technique of Giolitti and Cantoni (1966). Total viable counts were obtained on TGYE agar.

RESULTS AND DISCUSSIONS

The total viable counts obtained on the meat and fat surfaces of the striploin (Table 1) show that fairly high levels of contamination were present on the meat before vacuum-packaging though there was little difference between the levels on the two types of surface. A mixed flora was found, dominated by Gram-negative short rods which were mainly oxidase positive; the remainder were identified for the most part as M. thermosphactum and there were rather more of these organisms on the fat than on the meat surface. This flora could probably be regarded as fairly typical of meat at this stage of handling. After 8 weeks at 0-2° in evacuated packages, the flora changed to one comprising largely catalase-negative rods and cocci, with a higher proportion of M. thermosphactum on the fat than on the meat. Considerable numbers of Gram-negative rods were also present however and the actual numbers of these are shown in Table 2 based on the isolates identified. This meat was not further incubated to study the development of the spoilage flora, though the meat after 8 weeks smelled acidic and 4 of the pieces showed green discolouration. The pH and ERV values obtained from the samples showed very little change from those observed initially. The Gram-negative rods though not multiplying as rapidly as the lactic organisms nevertheless increased by a factor of x10 at least and it was suspected that these might play a part in the ultimate spoilage (and the shelf-life) of the product, since the lactic organisms did not appear able to hold them completely in check even under relatively unsuitable growth conditions (low O_2 tension and low temperature). In this experiment (and in some earlier work) a considerable numbers of the Gram-negative rods particularly from the fat surfaces were not pseudomonads, and were difficult to identify. Typically they were motile, oxidase-negative, fermentative (Hugh and Leifson, glucose) indole-negative methyl red-negative, citrate-negative, VP-positive, H_2S-negative, urease-negative and only

fermented lactose slowly with little gas formation.

The numbers and types of micro-organisms developing on the meat slices after opening and storing in air for up to 96 h following 3 and 5 weeks in a vacuum-package are shown in Table 3. On both these samples the numbers recovered on MGV which were mainly oxidase positive pseudomonads and VRB which were possibly Gram-negative fermentative rods, were high, indicating fairly high initial contamination. Storage in air resulted in fairly rapid multiplication until after 72-96 h numbers of these organisms (10^7-10^8/cm^2) were sufficient to cause typical aerobic spoilage, associated with increased pH value (>6.0) and lowered ERV. Samples from other weeks however did not show this, and although the numbers recovered on TGYE were high (10^8/cm^2), the numbers of pseudomonads and related organisms had not reached sufficient numbers to cause aerobic spoilage. The smell of the meat was still acidic though it had an unattractive appearance while the pH and ERV had not changed greatly. For these meat samples the initial numbers of Gram-negative rods tended to be low, emphasising the need for good hygiene and careful handling of the meat during preparation before vacuum-packaging, together with good temperature control at all stages if a reasonable shelf-life on opening is to be obtained. Where spoilage had not been brought about by pseudomonads, it was rather more difficult to assess potential shelf-life, and the appearance and smell of the meat is likely to determine its saleability. Certainly the microbial population increased to quite high levels within 2-4 days on all slices stored in air at 4-6° whether mainly lactic in type or otherwise.

The question whether potential pathogens such as species of *Salmonella* and *Clostridium* and *Staph. aureus* are a potential hazard in vacuum-packaged meat has not so far been answered by our work. Table 4 gives an indication of what

is likely to happen with Staph. aureus, where low numbers of one strain survived for 8 weeks though with decreasing numbers and higher numbers of 2 strains survived for at least 4 weeks. There was little evidence of rapid multiplication when the package was opened and the meat held at 15°, probably due to the fact that competing organisms multiplied rapidly while holding the staphylococci in check. Further work is in progress on this aspect of the study.

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TABLE I

MICROBIAL CHANGES ON STORED PACKAGED MEAT

	Mean Log counts/cm^2 after 12d at 4° on TGYE	
	Before Packaging	After 8 weeks at $0-2^{\circ}$
Meat Side	5.18	7.68
	Range (6 samples) 4.04-6.10	7.48-7.82
Fat Side	5.26	7.28
	Range (6 samples) 3.72-6.68	6.60-7.49

	Changes in flora as %	
Meat Side (120 isolates)	Pseudomonads gp II/Achromobacter/Alcaligenes 55.8; Moraxella spp 30.0; Ps gp I 7.5; Microbacterium thermosphactum 6.7.	Gram+, catalase- rods or cocci, 90.0; Microbacterium thermosphactum 5.9; Moraxella spp 3.3; Acinetobacter spp 0.8.
Fat Side (120 isolates)	Ps gp II/Achromobacter/Alcaligenes 51.7; Moraxella spp 26.7; Microbacterium thermosphactum 13.3; Ps gp I 7.5; G-, fermentative 0.8	Gram+, catalase- rods or cocci, 63.8; Microbacterium thermosphactum 20.2; G-, fermentative 10.9; Ps gp I 0.8, Ps GPII/Achr./Alcal. 0.8; Moraxella spp 0.8; Acinetobacter spp 0.8

TABLE 2

CHANGES IN NUMBERS OF GRAM-NEGATIVE RODS ON STORED PACKAGED MEAT (MEAN LOG/cm^2)

		Before packaging	After 8 weeks at 0-2°
Meat Side		5.16	6.45
	Range	4.04-6.05	6.09-7.00
Fat Side		5.19	6.41
	Range	3.62-6.68	5.78-6.69

TABLE 3

CHANGES IN FLORA ON MEAT STORED AEROBICALLY AT 4-6° AFTER AGEING IN VACUUM-PACKAGES FOR 3 AND 5 WEEKS AT 0-2°

Period of ageing	Period of aerobic storage (hr)	Log counts/cm^2 on TGYE 4°	TGYE 15°	MGV	VRB	pH	ERV(ml)
3 weeks	0	7.67[1]	7.65	5.23	4.97	-	-
		5.67[2]	5.62	3.92	3.26	5.80	-
	6	5.50	5.51	3.09	2.79	5.80	49.5
	12	6.23	6.32	4.63	4.09	6.15	37.5
	24	5.61	5.66	3.90	3.15	6.10	31.5
	36	6.44	6.51	4.55	4.26	6.20	38.5
	48	6.65	6.79	5.03	4.11	6.40	31.0
	54	7.51	7.50	6.01	5.32	5.80	39.0
5 weeks	0	8.18[1]	8.25	6.00	<1.70	-	-
		>5.00[2]	>5.00	4.39	4.30	6.05	43.0
	12	5.85	5.88	3.26	4.16	6.20	39.0
	24	7.33	7.41	5.87	5.30	6.35	23.0
	36	6.54	6.64	4.60	4.53	5.95	32.0
	48	7.54	7.57	5.77	5.54	5.85	33.0
	54	7.09	7.11	6.12	3.76	6.20	39.0
	72	8.48	8.47	7.22	>5.00	6.45	17.0
	96	8.93	8.86	8.86	>5.00	7.00	10.0

[1] Top of meat block.

[2] Surface of slice.

TABLE 4

SURVIVAL OF STAPH. AUREUS ON VACUUM-PACKAGED MEAT

Level of inoculum/g	Survival of Staph. aureus after (weeks) at 1-2°							
	1	2	3	4	5	6	7	8
3*	+	+	+	+	+	+	+	+
10**	+	+	-	-				
300*	+	+	+	+	+	+	+	+
1000**	+	+	+	+				

* # MP136 ** # MP1 + = viable cells present

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SUMMARY

The objective of this work was (i) to study the changes in bacterial contamination which take place on meat when it is vacuum-packaged and held at 0-2°; (ii) to find how the changes continue after the meat has been sliced and stored in air after vacuum-packaging and (iii) to investigate the survival of potentially dangerous bacteria on the meat during storage at 0-2° and after opening the package. The results indicate that (i) the contaminants on meat before vacuum-packaging do not all disappear during storage, even though the population changes to mainly lactic rods and cocci. Some aerobic potential spoilage organisms survive and multiply in the vaccum-package; (ii) Several of the meat slices when stored at 4-6° after holding in vacuum-packages at 0-2° were spoiled by a typical aerobic group of bacteria; whereas others appeared to be spoiled by lactic bacteria; (iii) The hygiene of butchering and temperature control during preparation of the meat is very important; (iv) Two strains of Staphylococcus aureus were capable of surviving in the vacuum-package for at least 8 weeks.

Further work is needed to study the spoilage activities of the bacteria on the meat after vacuum-packaging, and the survival and possible multiplication of potential pathogens after opening the package.

DIE AUF FLEISCH AEROBISCH ENTWICKELNDEN MIKROFLORA NACH REIFUNG IM VAKUUMBEUTEL

ZUSAMMENFASSUNG

Die Untersuchungsziele waren: (i) die Veränderungen bacterieller Verwesung zu studieren, die beim Fleisch nach Verpackung im Vakuumbeutel und Lagerung bei 0-2° vorkommen; (ii) die eventuellen Veränderungen zu bestimmen, die sich nach dem Schneiden des Fleisches und dessen Luftlagerung nach Vakuumverpackung fortsetzen; und (iii) das Weiterleben auf dem Fleisch von vielleicht gefährlichen Bakterien während Lagerung bei 0-2° und auch nach dem Aufmachen des Beutels zu untersuchen. Die Ergebnisse zeigen dass: (i) nicht alle Verseuchungsagenzien verschwinden, die auf dem Fleisch vor der Verpackung vorhanden sind, selbst wenn sie sich zum grössten Teil zu Angehörigen der Gattung Lactobacillaceae verändern; einige aerobischen potentiellen Verwesungsorganismen fortleben und vermehren sich im Vakuumbeutel; (ii) mehrere Fleischscheiben, nach dem sie im Vakuumbeutel bei 0-2° gehalten und dann bei 4-6° gelagert wurden, kamen zu Verwesung, verursacht durch eine typische aerobische Bakteriengruppe; andere dagegen kamen zu Verwesung verursacht durch Lactobazillen; (iii) die Gesundheitsmassnahmen während des Schlachtens und der Temperaturkontrolle sind sehr wichtig; (iv) zwei Stämme der Gattung Staphylococcus aureus konnten mindestens 8 Wochen im Vakuumbeutel weiterleben.

Man braucht die Verwesungsaktivität der auf dem Fleisch nach Vakuumverpackung vorhandenen Bakterien weiter zu studieren, auch das Weiterleben und mögliche Vermehrung von potentiellen Pathogenen nach dem Aufmachen des Beutels.

LA MICRO-FLORE QUI SE DÉVELOPPE D'UNE MANIÈRE AÉROBIE SUR LA VIANDE APRÈS LA MATURATION SOUS VIDE

Le but de ce travail était: (i) d'étudier les mutations de contamination bactérienne qui se produisent sur la viande après qu'on l'a mise en sachets sous vide et l'a tenue a 0-2°; (ii) d'établir comment continuent les mutations après le découpage de la viande et son entreposage en l'air après l'emballage en sachets sous vide; et (iii) d'étudier la survivance sur la viande de bactéries potentiellement dangereuses pendant l'entreposage a 0-2° et aussi après que l'emballage a été ouvert. Les résultats montrent que: (i) les agents viciateurs ne disparaissent pas tous pendant l'entreposage bien qu'ils se changent pour la plupart aux membres du genre lactobacillaceae. Quelques organismes aérobies de décomposition potentielle survivent et se multiplient en sachets sous vide; (ii) après que quelques-unes des lêches de viande ont été tenues a 0-2° en sachets sous vide et qu'elles ont été entreposées à 4-6°, elles furent gâtées par un groupe aérobie typique de bactéries; d'autre part il apparaissait que d'autres lêches avaient été gâtées par lactobacilles; (iii) L'hygiène de boucherie et de contrôle de la température pendant la préparation de la viande est très importante; (iv) deux espèces de Staphylococcus aureus ont pu survivre en sachets sous vide pendant au moins 8 semaines.

Il est nécessaire d'étudier plus étroitement l'activité nuisible des bactéries sur la viande après la mise en sachets sous vide et la survivance et la multiplication éventuelle de microbes pathogènes en ouvrant l'emballage.

Аэробный рост микрофлоры на говядине после старения в вакууме

КРАТКОЕ ИЗЛОЖЕНИЕ

Цель данной работы заключалась в том, чтобы (а) изучать перемены в бактерийном заражении происходящие на мясной туше тогда, когда, упаковав её в вакууме, держат на температуре в пределах 0-2°; (б) узнать, каким образом эти перемены продолжаются, когда, после упаковки мясной туши в вакууме, она разрезалась на ломти и хранилась в воздухе; (в) исследовать, каким образом потенциально вредные бактерии находящиеся на туше переживают при хранении на температуре 0-2° и после вскрытия упаковки. Результаты произведённого исследования указывают на то, что (а) не все находящиеся на туше до упаковки в вакууме агенты заражения исчезают во время хранения, хотя происходит перемена в их составе, большей частью к молочным палочкам и коккам - при упаковке в вакууме переживают и множатся некоторые аэробные организмы имеющие способность производить порчу; (б) некоторые из ломтей туши, при хранении на температуре 4-6° после того, как их держали в вакууме на температуре 0-2°, испортила типичная группа аэробных бактерий, тогда как другие из них испортили, по-видимому, молочные бактерии; (в) очень важны соблюдение гигиены при разделке туши, управление температурой при подготовке туши; (г) при упаковке в вакууме проявляли способность переживать по крайней мере 8 недель два штамма Стафилококка золотистого.

Требуется дальнейшая работа по изучению как портящей деятельности бактерий по туше после упаковки её в вакууме, так и переживания и возможного размножения потенциальных патогенов после вскрытия упаковки.